Nanomaterial Toxicity Testing in the 21st Century: Use of a Predictive Toxicological Approach and High-Throughput Screening

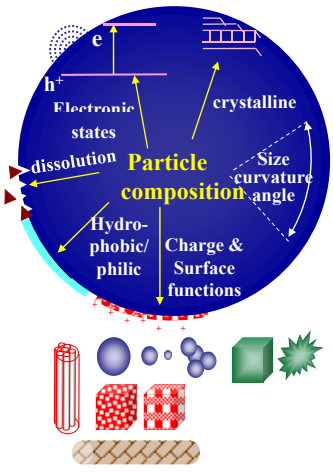
André Nel M.B.,Ch.B; Ph.D
Professor of Medicine and Chief of the Division of NanoMedicine at UCLA

Director of the NSF- and EPA-funded Center for the Environmental Implications of Nanotechnology (UC CEIN)

Director of the NIEHS-funded Center for NanoBiology and Predictive Toxicology

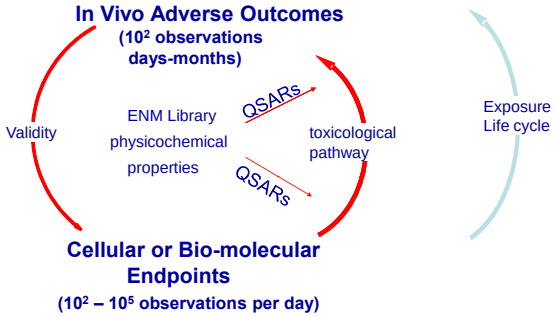
This materials is based on work supported by the National Science Foundation and Environmental Protection Agency under Cooperative Agreement # NSF-EF0830117. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation or the Environmental Protection Agency.

Large number of materials with novel physicochemical properties



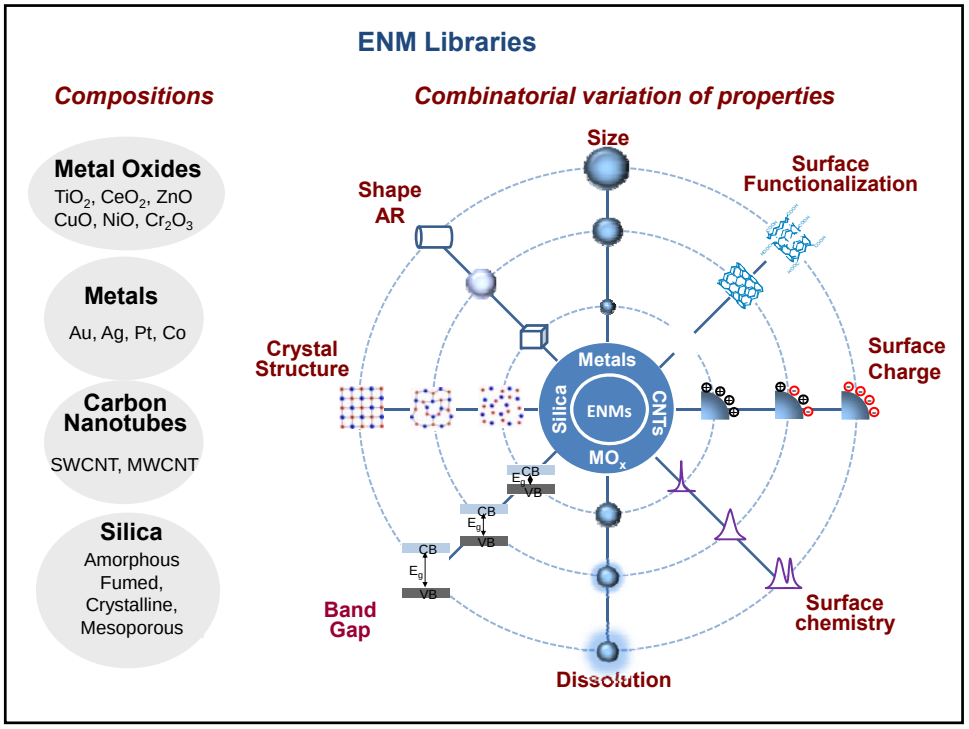
US National Academy of Science Report (2007)

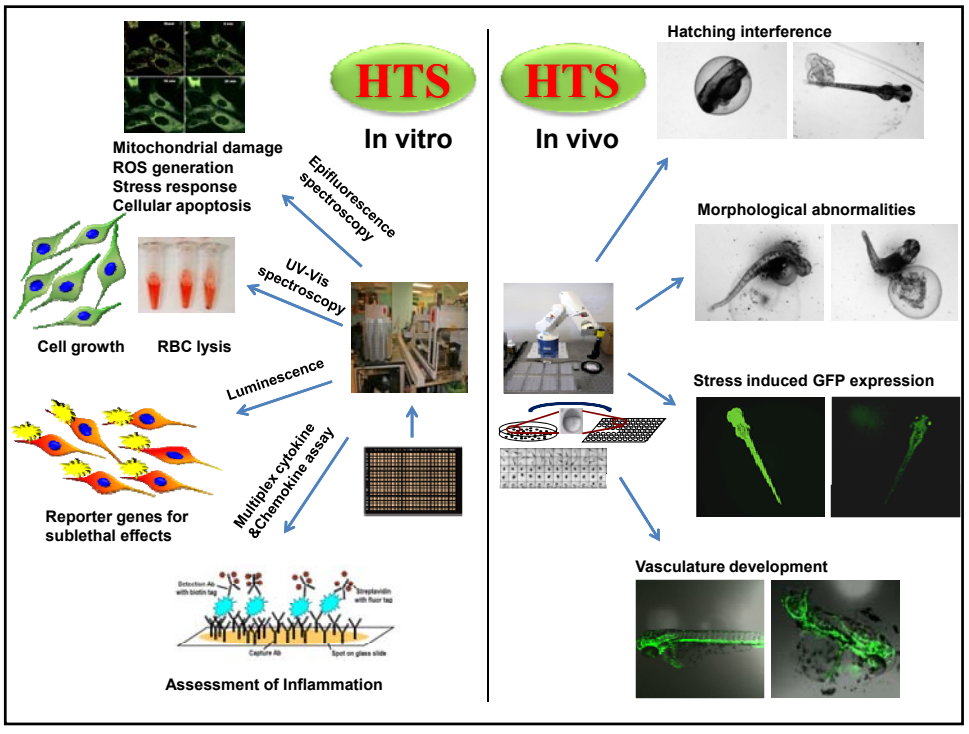
- Wide coverage of toxicants
- Robust scientific platform for screening
- Predictive tests utilizing toxicity mechanisms
- High throughput screening
- Connectivity to *in vivo*



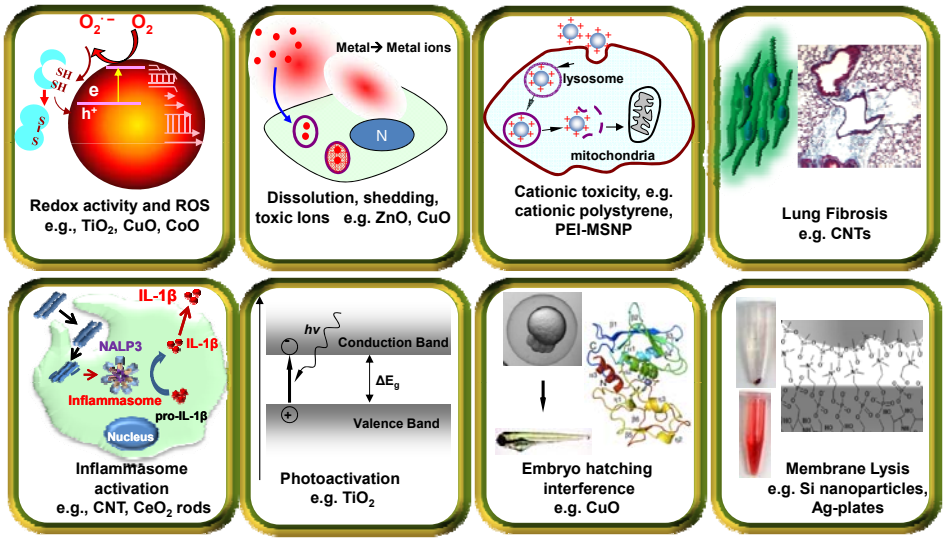
Meng et al. ACS Nano. 2009
Nel et al. Accounts Chem Res, 2012

http://www.nap.edu/catalog.php?record_id=11970


ENM Libraries
Compositions
Combinatorial variation of properties
Metal Oxides
TiO_2, CeO_2, ZnO
CuO, NiO, Cr_2O_3
Metals
Au, Ag, Pt, Co
Carbon Nanotubes
SWCNT, MWCNT
Silica
Amorphous
Fumed,
Crystalline,
Mesoporous
Size
Surface Functionalization
Shape
AR
Crystal Structure
Surface Charge
Band Gap
Surface chemistry
Dissolution
Metals
Silica
ENMs
CNTs
MO_x
CB
E_g
VB


HTS
In vitro
HTS
In vivo
Mitochondrial damage
ROS generation
Stress response
Cellular apoptosis
Epifluorescence spectroscopy
UV-Vis spectroscopy
Cell growth
RBC lysis
Luminescence
Multiplex cytokine &Chemokine assay
Reporter genes for sublethal effects
Assessment of Inflammation
Hatching interference
Morphological abnormalities
Stress induced GFP expression
Vasculature development

Mechanistic Toxicological Pathways in Cells that can be used for HTS & Predictive Toxicological Modeling



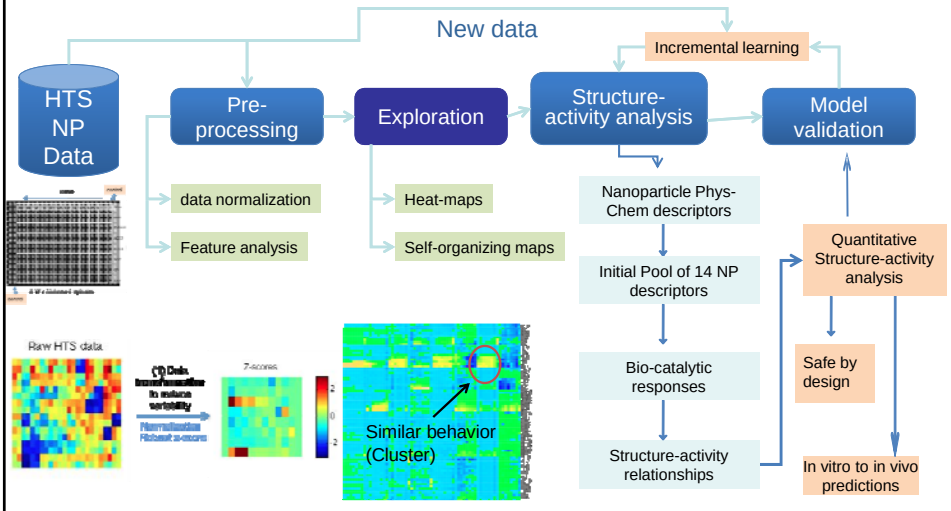
Nel et al. Nature Materials. 2009
Xia et al, ACS Nano, 2008
Xia et al. ACS Nano. 2011

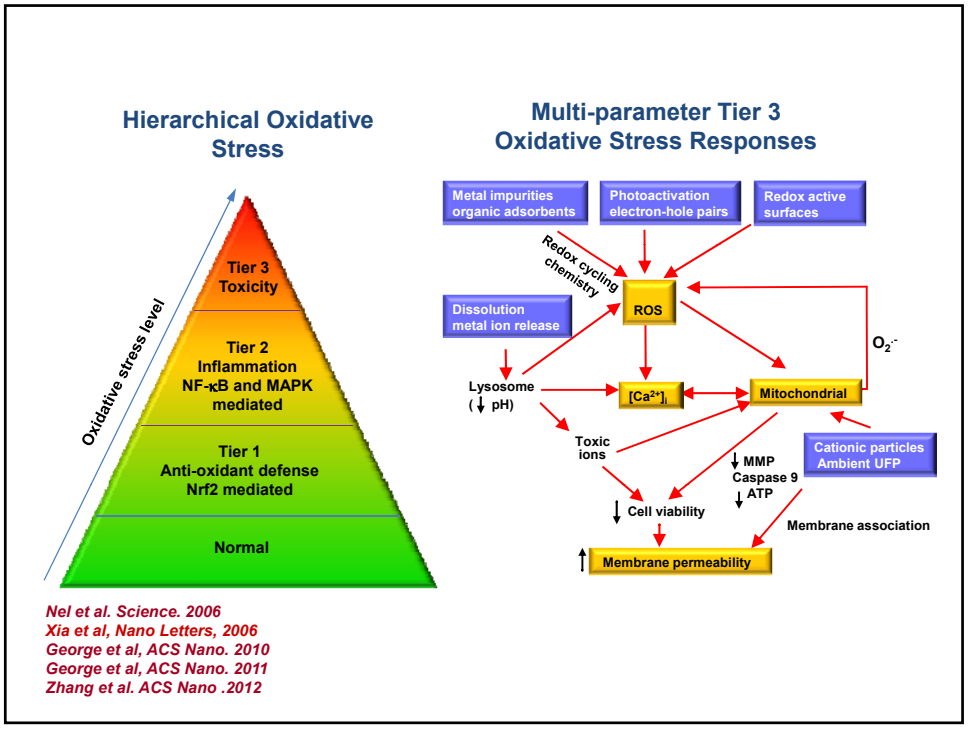
George et al. ACS Nano. 2010
George et al. ACS Nano. 2011
George et al JACS 2011

Lin et al. ACS Nano. 2011
Xia et al ACS Nano. 2009
Zhang et al ACS Nano 2011

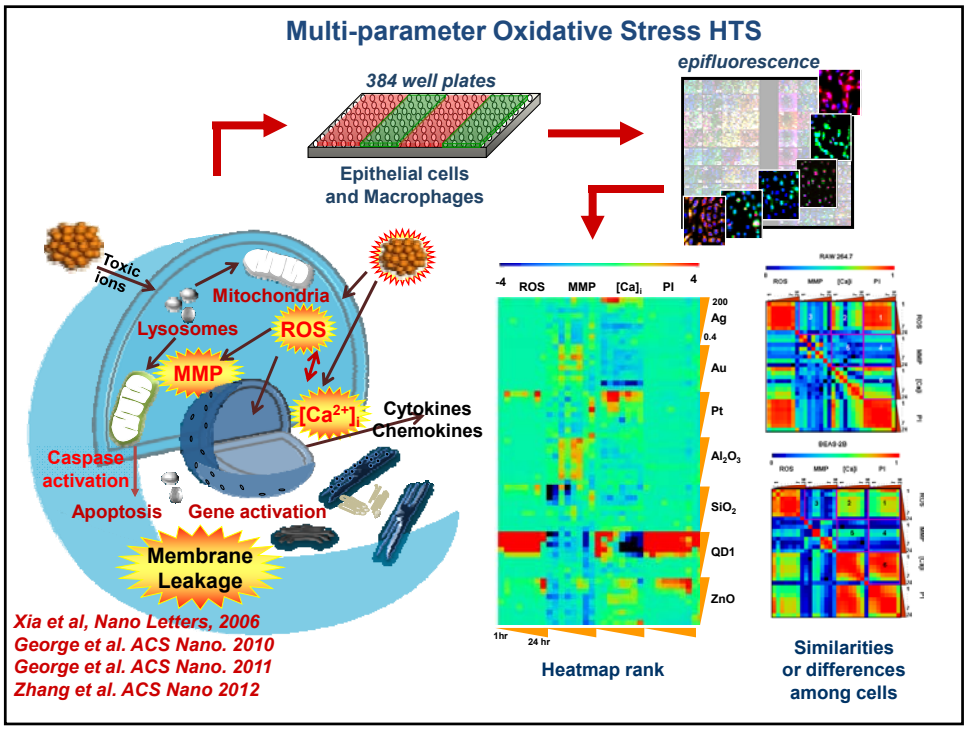
Wang et al. ACS Nano. 2010
Wang et al ACS Nano. 2011

Data collection, exploration, SAR modeling



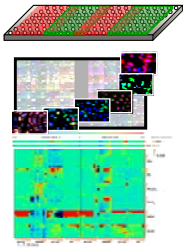


Nel et al. Science. 2006
Xia et al, Nano Letters, 2006
George et al, ACS Nano. 2010
George et al, ACS Nano. 2011
Zhang et al. ACS Nano .2012



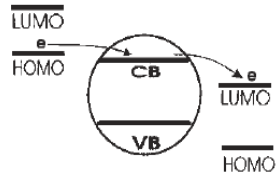
Xia et al, Nano Letters, 2006
George et al. ACS Nano. 2010
George et al. ACS Nano. 2011
Zhang et al. ACS Nano 2012

Permissible Energy Levels allow Electron transfer between NPs and Redox-active substances in a Biological environment

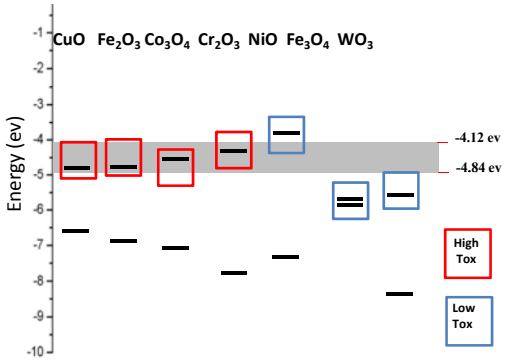


MO_x NP descriptors

- Band gap energy
- NP Primary Size (nm)
- Metal atomic number
- Dissolution characteristics

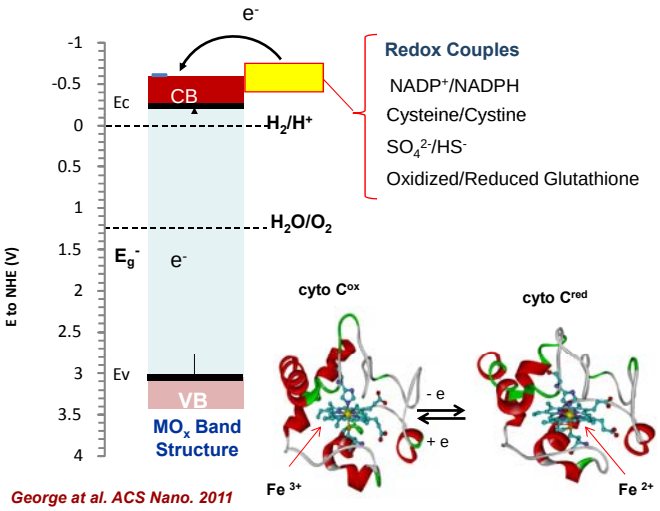


Xu and Schoonen. Amer Minirologist . 2000



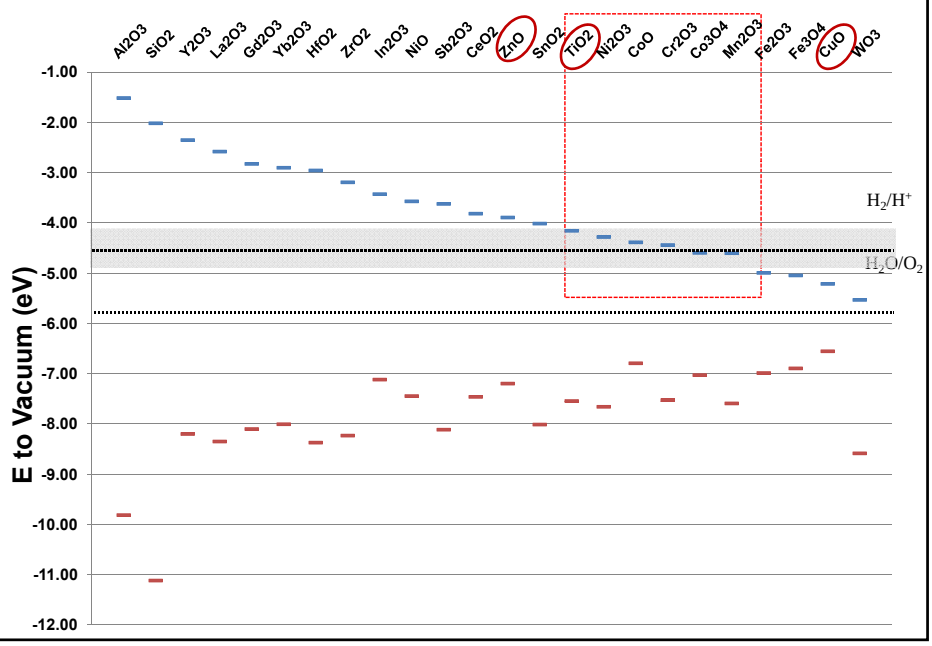
Burello. Nanotoxicology, 2010

MOx Conduction band gap energy level as a possible Electron Acceptor for Cytochrome c Redox shuttling

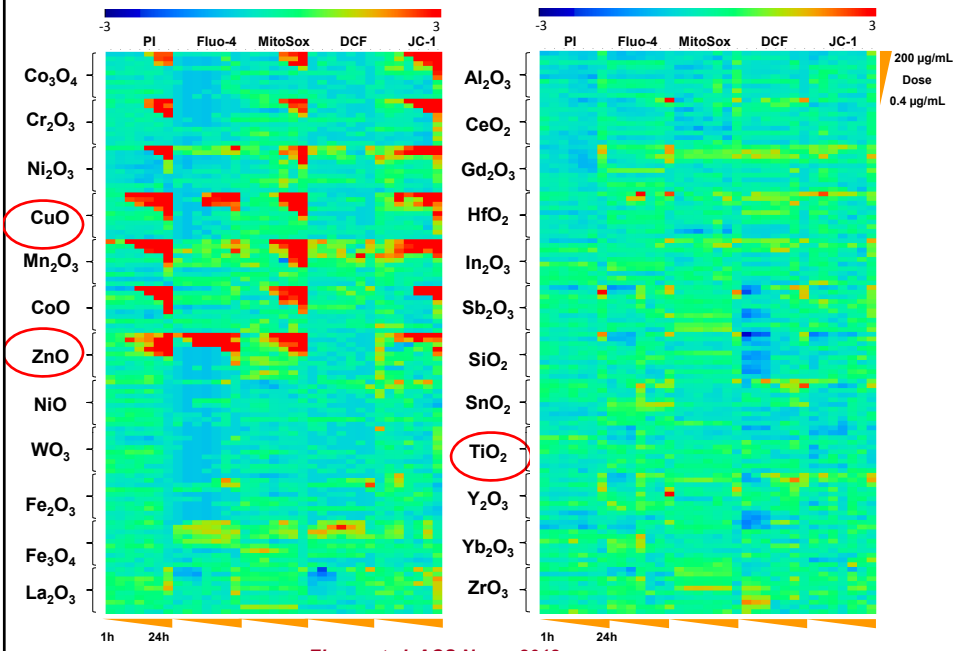


George at al. ACS Nano. 2011
Liu et al. Small. 2011
Rallo et al. EST. 2011
Zhang et al. ACS Nano. 2012.

Prediction of MOx Toxicity based on Bandgap Energy

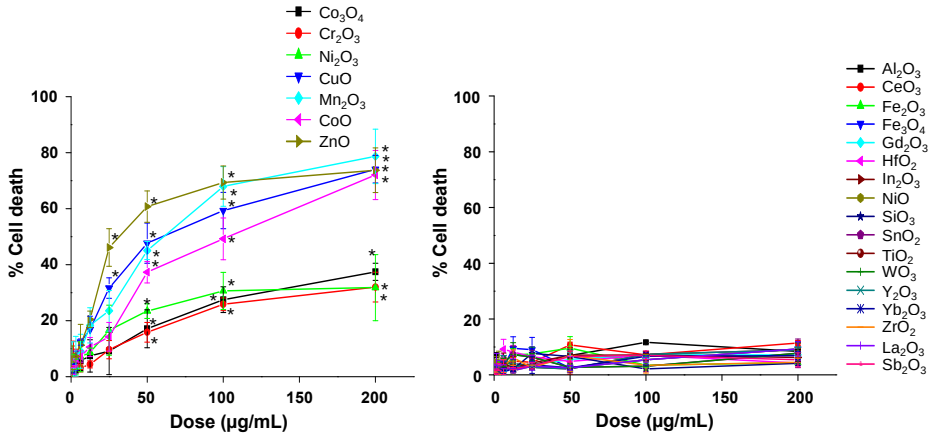


Heatmap display MOx HTS in RAW cells



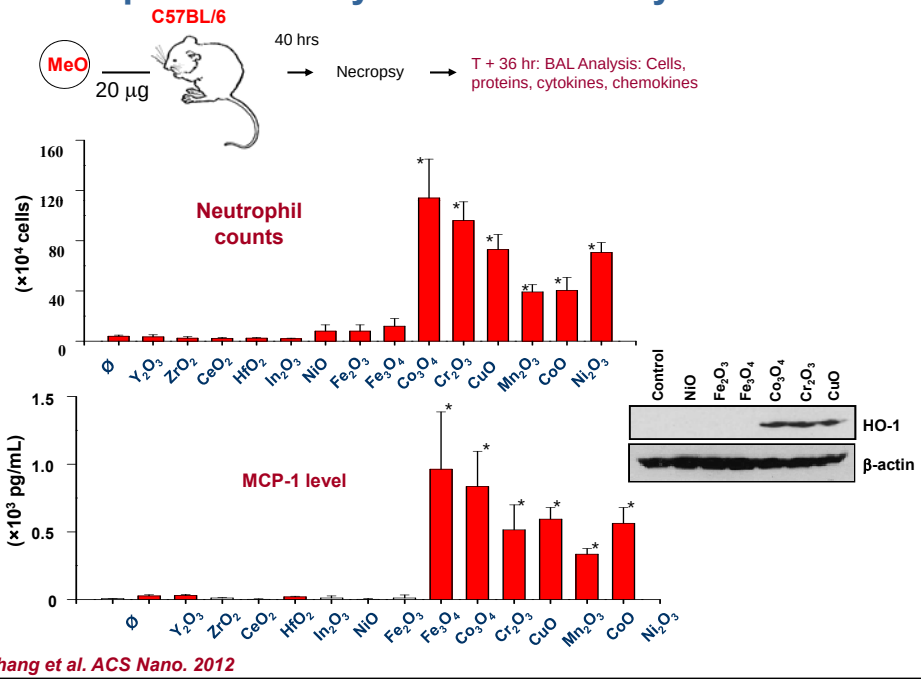
Zhang et al. ACS Nano. 2012

LDH assay in Bronchial Epithelial Cell Line



Zhang et al. ACS Nano. 2012

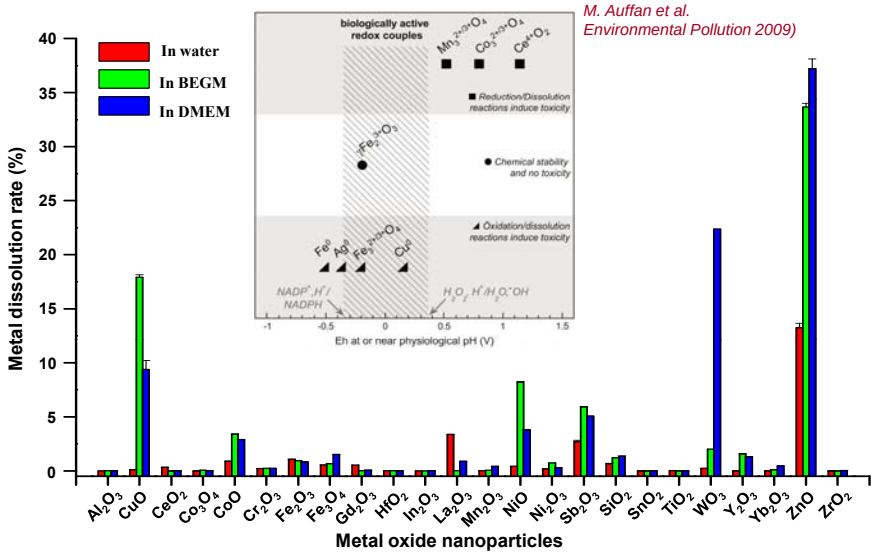
Comparative analysis of Pulmonary Inflammation



Zhang et al. ACS Nano. 2012

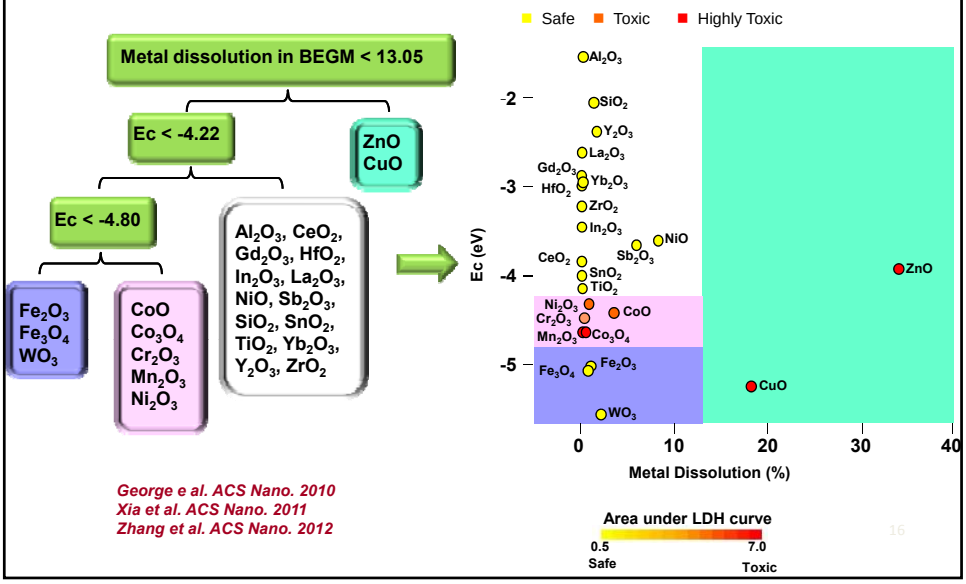
MOx Nanoparticle Dissolution: Oxidation and Reduction Dissolution Reactions in Response to Biologically Active Redox Couples

M. Auffan et al. Environmental Pollution 2009)



Zhang et al. ACS Nano. 2012

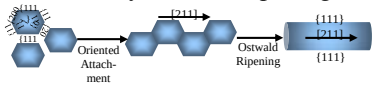
Regression Tree for MOx Toxicity: Combination of Conduction Energy plus dissolution

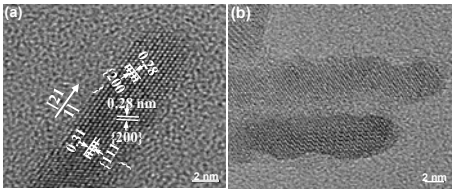


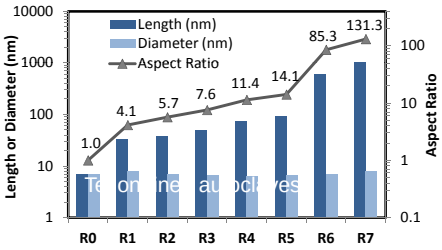
George e al. ACS Nano. 2010
Xia et al. ACS Nano. 2011
Zhang et al. ACS Nano. 2012

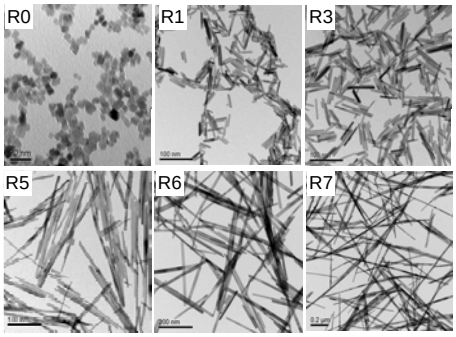
Synthesis of a Ceria Nanowire Library to Study shape-dependant toxicity

- Synthesized by hydrothermal technique
- Aspect ratio can be finely tuned from 1 to >200
- Growth by oriented attachment followed by Ostwald ripening







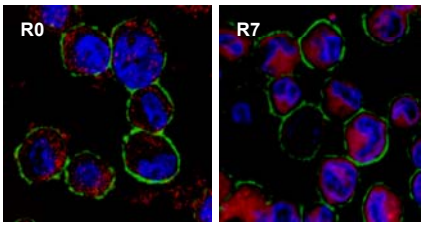


Ji, Xia, Wang, Nel, Zink ACS Nano 2012

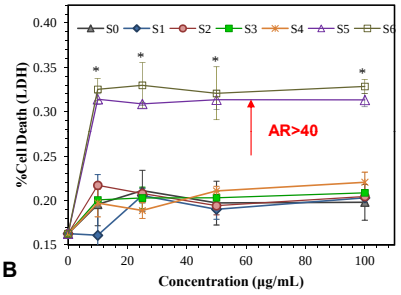
Ceria Nanowire Toxicity in THP-1

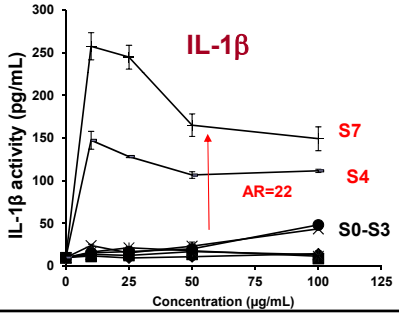
No Cathepsin B release

Cathepsin B release



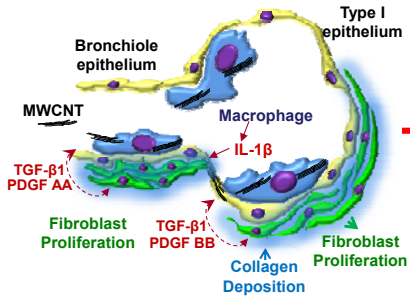
PI uptake - cytotoxicity



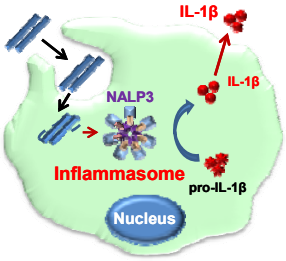


Ji, Xia, Wang, Nel, Zink ACS Nano 2012

Predictive Paradigm for CNT and Long aspect Ratio Toxicity



The epithelial-mesenchymal trophic cell unit



Inflammasome activation

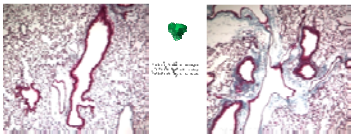
In vitro

Dispersed vs. Non-Dispersed MWCNT

Macrophage → ↑ IL-1β → **Epithelial cells** → ↑ TGF-β1

In vivo

Non-Dispersed MWCNT | Dispersed MWCNT



↑Fibrosis

BAL Fluid Biomarkers

Day 1: ↑ IL-1β

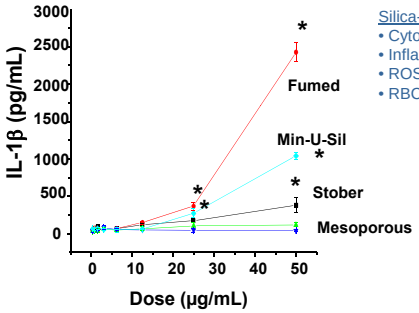
Day 21: ↑ TGF-β1
↑ PDGF-AA

Wang et al. ACS Nano. 2010
Wang et al. ACS Nano. 2011

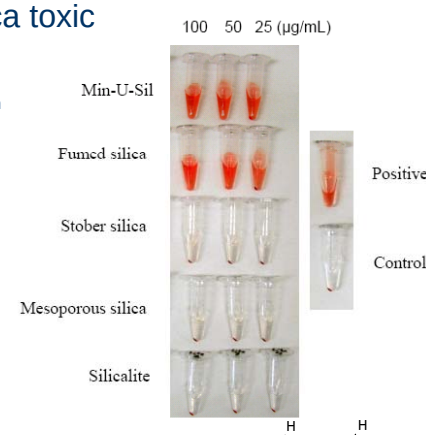
19

High Content Screening to Elucidate Silica Toxicity- MSNP does not show the structural features that make silica toxic

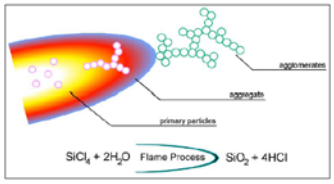
Haiyuan Zhang, Jeff Brinker, Jeff Zink



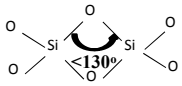
Silica-induced
- Cytotoxicity
- Inflammation
- ROS
- RBC Lysis



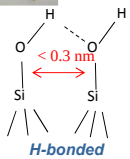
Fumed Silica



Strained ring



2 & 3-member rings



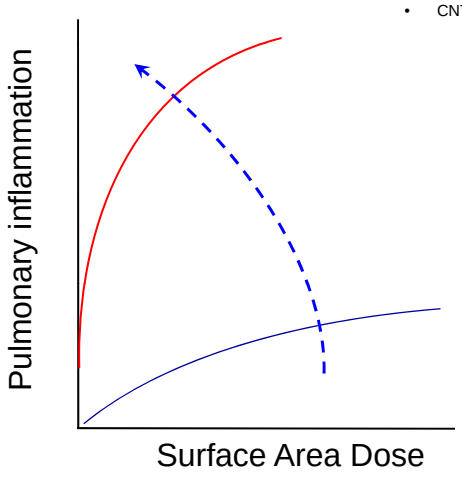
H-bonded silanols

Pulmonary Toxicity Ranking

UCLA Center for NanoBiology and Predictive Toxicology

SARs predicting high pulmonary inflammation potential:

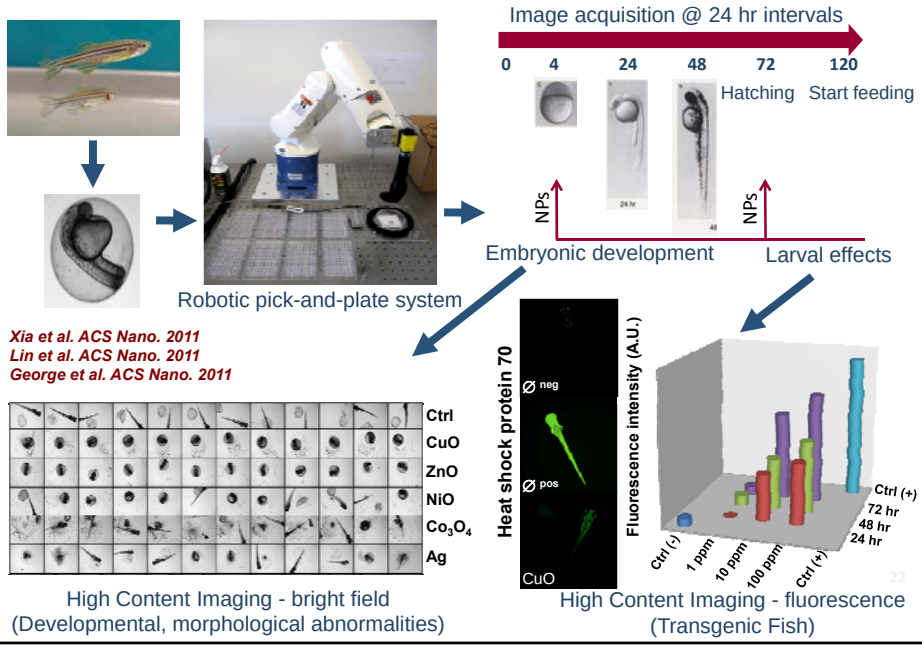
- Crystalline and fumed Si (OH density)
- CuO, ZnO (dissolution)
- Co, Ni, Cr etc (band gap)
- LAR MOx's (lysosomal, NALP3)
- CNT's (LAR and fibrogenic effects)



SARs predicting low pulmonary inflammation potential:

- TiO_2 and CeO2 spheres
- Au (non-functionalized)
- Carbon black
- Amorphous Si

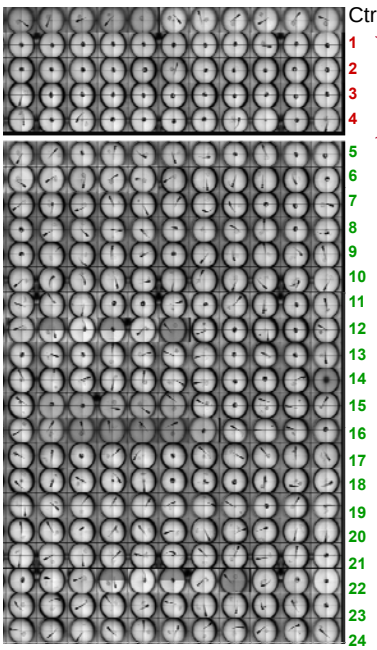
Zebrafish HTS platform → automated imaging of developmental abnormalities and transgenic responses



High Content Imaging - bright field (Developmental, morphological abnormalities)

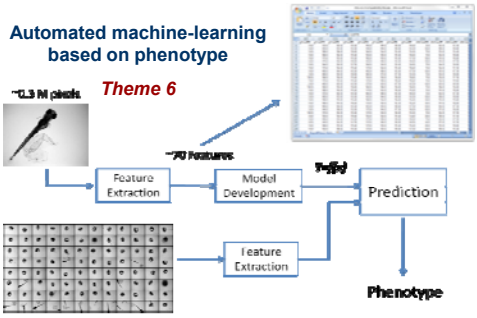
High Content Imaging - fluorescence (Transgenic Fish)

Zebrafish HTS → hazard ranking on 24 MOx's

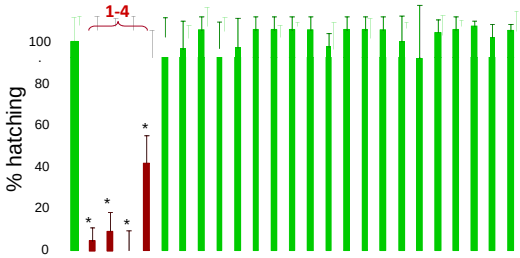


Automated machine-learning based on phenotype

Theme 6

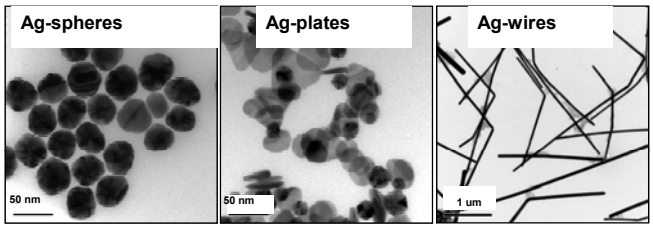


Liu et al. PLOS One. In Press

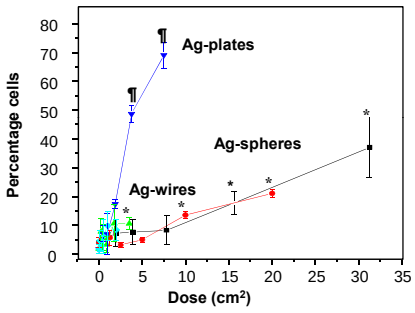


Lin et al. In preparation

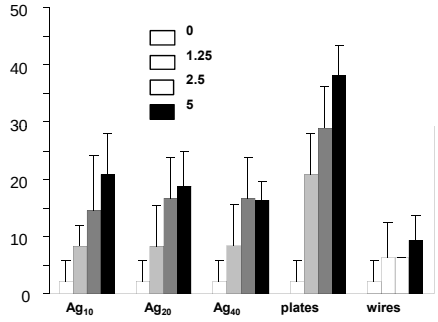
Shape plays a role in Nano-Ag toxicity in Zebrafish By a Paradigm other than Ag-ion shedding



Fish Cell death (% PI uptake)

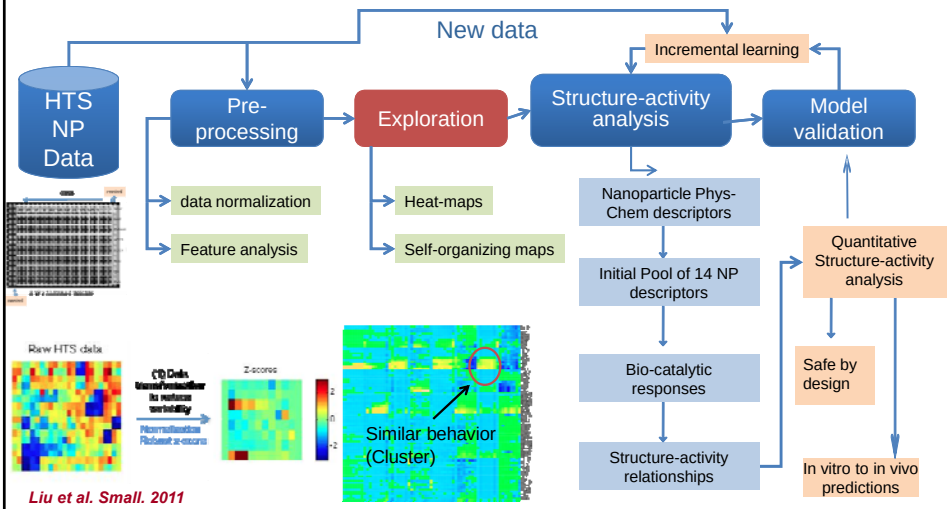


% Embryo hatching ↓



George et al. ACS Nano. 2012

Data collection, exploration, SAR modeling

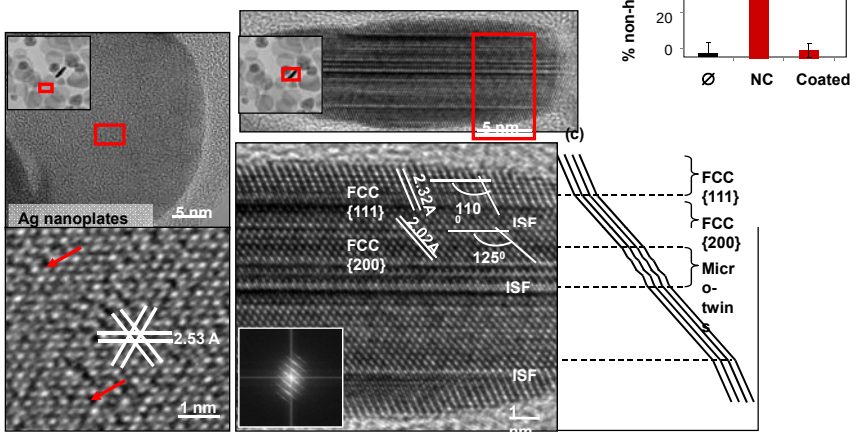


Liu et al. Small. 2011
Rallo et al. EST. 2011
Damoiseaux et al. Nanoscale. 2011

Passivation of surface defects on the Ag Nanoplate Surface Decreases Toxicity

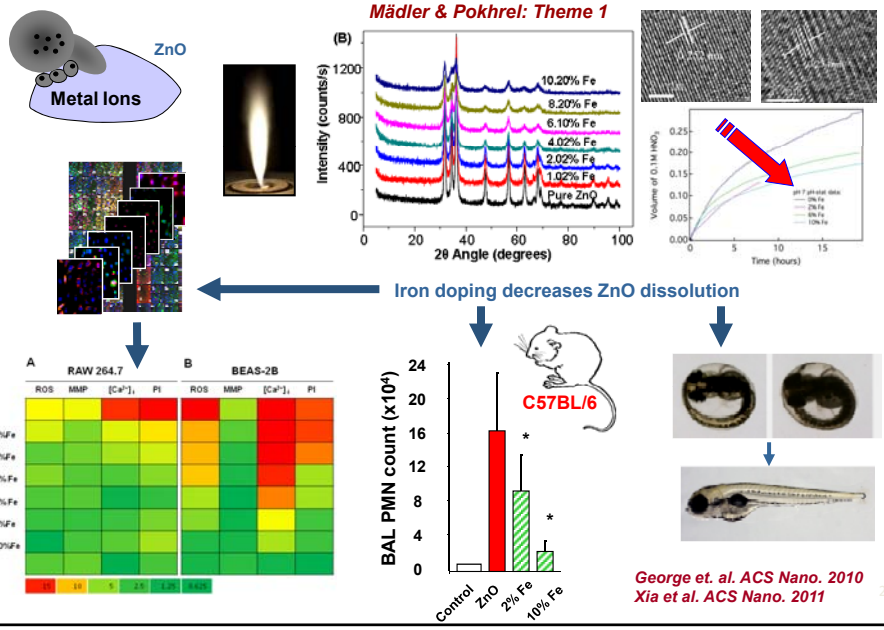
George et al. ACS Nano. 2012

Cysteine coating



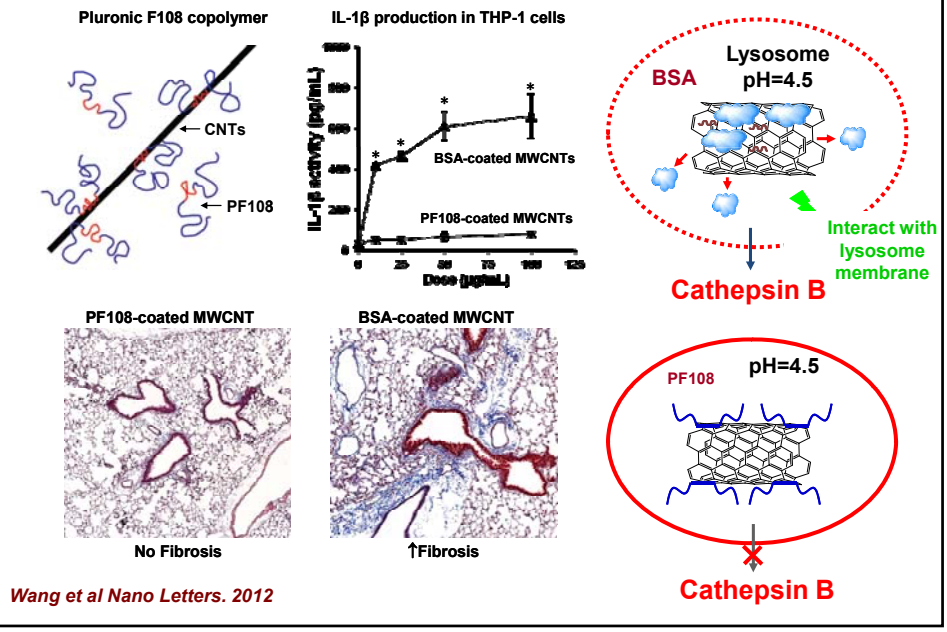
Safer design principle for nano-ZnO by using iron doping

Mädler & Pokhrel: Theme 1



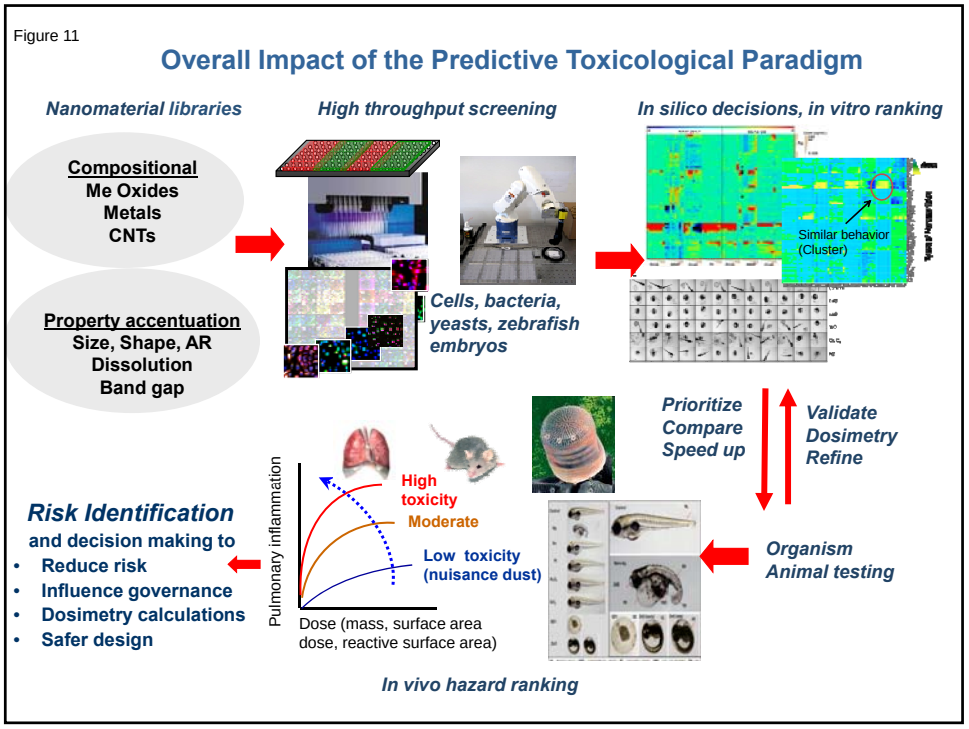
George et. al. ACS Nano. 2010
Xia et al. ACS Nano. 2011

Pluronic-treated SWCNT do not induce IL-1β: failure to engage the inflammasome

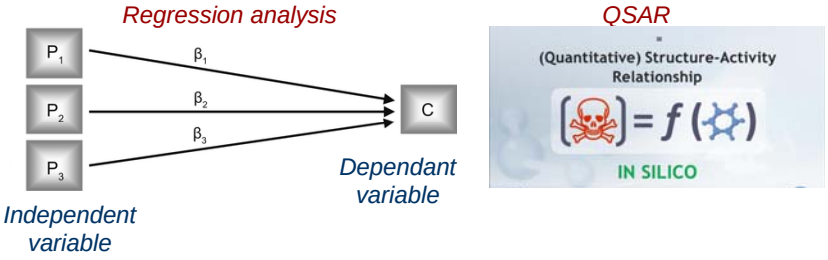


Wang et al Nano Letters. 2012

Figure 11

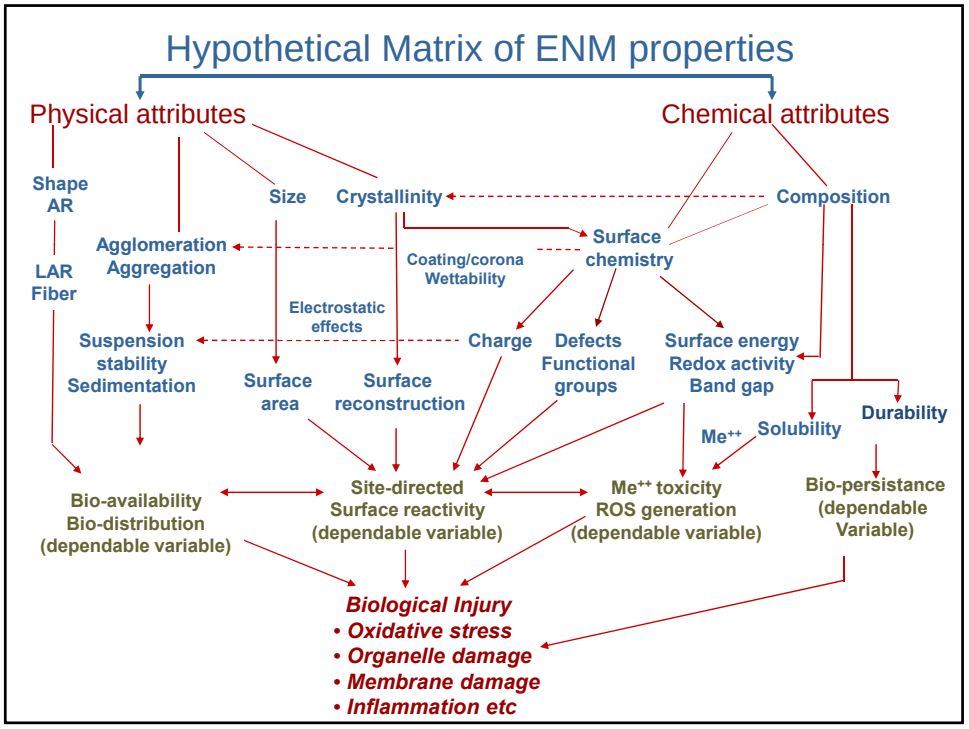


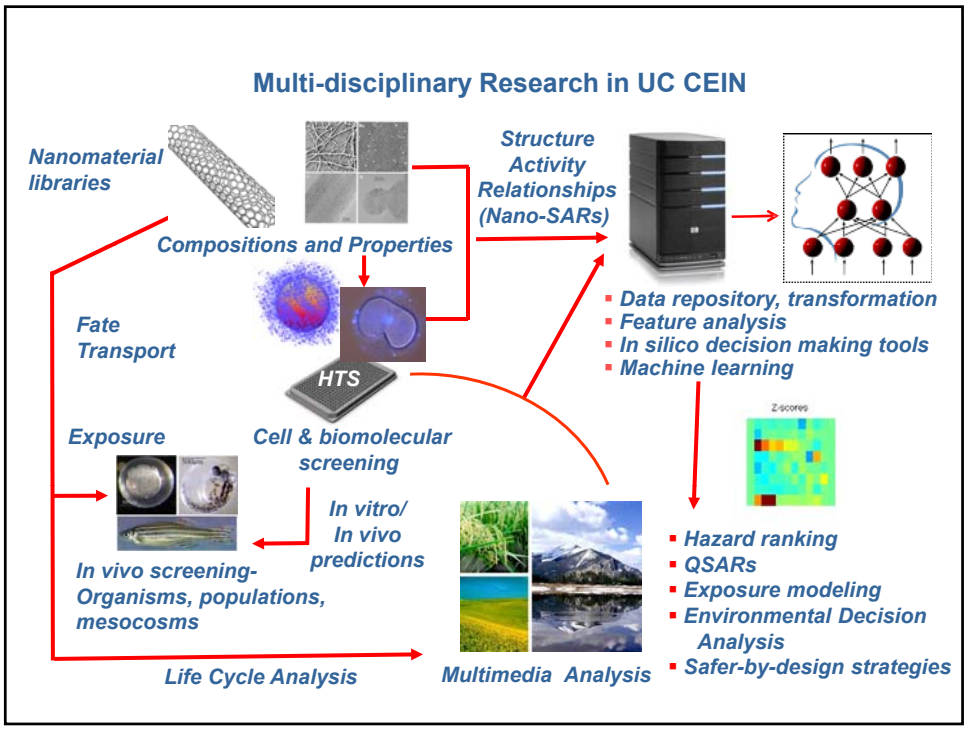
Quantitative Structure-Activity Relationships in Nanomaterial Toxicity



Quantitative SARs (QSARs) are regression analysis models in which fundamental material physicochemical descriptors are employed to generate quantitative models for correlating and predicting injury responses resulting from exposure to ENMs. Fundamental descriptors are essential since dependable variables (most proximate physicochemical properties associated with injury) may be determined by a series of independent variables in a hierarchically structured matrix. The ultimate goal of QSAR models is to predict the activity of new ENMs and also establish the range of ENM descriptors suitable for the design of safe ENMs.

Liu et al. Small. 2011
Rallo et al. EST. 2011


Hypothetical Matrix of ENM properties
Physical attributes
Chemical attributes
Shape
AR
LAR
Fiber
Size
Crystallinity
Composition
Agglomeration
Aggregation
Coating/corona
Wettability
Surface
chemistry
Electrostatic
effects
Suspension
stability
Sedimentation
Charge
Defects
Functional
groups
Surface energy
Redox activity
Band gap
Surface
area
Surface
reconstruction
Durability
Me++
Solubility
Bio-availability
Bio-distribution
(dependable variable)
Site-directed
Surface reactivity
(dependable variable)
Me++ toxicity
ROS generation
(dependable variable)
Bio-persistance
(dependable
Variable)
Biological Injury
• Oxidative stress
• Organelle damage
• Membrane damage
• Inflammation etc


Multi-disciplinary Research in UC CEIN
Nanomaterial
libraries
Compositions and Properties
Structure
Activity
Relationships
(Nano-SARs)
Data repository, transformation
Feature analysis
In silico decision making tools
Machine learning
Fate
Transport
HTS
Exposure
Cell & biomolecular
screening
In vitro/
In vivo
predictions
In vivo screening-
Organisms, populations,
mesocosms
Life Cycle Analysis
Multimedia Analysis
Hazard ranking
QSARs
Exposure modeling
Environmental Decision
Analysis
Safer-by-design strategies